

Materials List for:

# Analysis of the Epithelial Damage Produced by *Entamoeba histolytica* Infection

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## Materials

Name	Company	Catalog Number	Comments
<i>Entamoeba histolytica</i> HM1:IMSS, Clone A	IMSS Hospital, Mexico	Without/number	Virulent trophozoites <sup>18</sup>
TYI broth	Becton, Dickinson and Company Merck Merck Merck J.T. Baker Reproquifin SIGMA-Aldrich SIGMA-Aldrich	211862 K35625437 626 21578 4873 3252-01 CAS 50-81-7 C7880 F-5879	3.45% BBL Biosate peptone 58 mM glucose 39 mM NaCl 5 mM KH <sub>2</sub> PO <sub>4</sub> 6.5 mM K <sub>2</sub> HPO <sub>4</sub> 16.3 mM ascorbic acid 8.1 mM L-cysteine 0.1 mM ferric ammonium citrate, adjust pH 6.8 <sup>19</sup>
Bovine serum adult	Microlab , Labs., Mex.	SU146	Use at 10% and inactivated to 56 °C for 30 min
Diamond vitamin mixture- Tween 80	In vitro	SR-07	Use at 3%
Penicillin	Lakeside, Méx.	34564SSA IV	0.5 IU/ml
Streptomycin	Lakeside, Méx.	75757SSA IV	35 µg/ml
Pyrex 15 ml screw cap culture tubes with PTFE lined phenolic caps	Corning-Pyrex	9826-16X	16 x 125 mm, capacity 15 ml and caps fabricated from special formula resistant to effects of temperature
Cell culture plates, 6-Well	Corning-Costar	3516	Sterile plates, well diameter 34.8 mm and growth area 9.5 cm <sup>2</sup> . Rings on lid prevent cross-contamination
25 cm <sup>2</sup> cell culture flask	Corning-Costar	430168	Canted neck flasks
MDCK (Madin Darby canine kidney) type I	American Type Culture Collection	CCL34	Kidney epithelial cells grown between the 60 <sup>th</sup> and 90 <sup>th</sup> passage
DMEM medium	Gibco	12800-017	Dulbecco's Modified Eagle Medium with high glucose.
Neonate Calf Serum	In vitro	S-02	Use at 10%.
Penicillin/Streptomycin mixture	In vitro	A-01	Stock solution 10,000 U/µg/ml
Insulin	AMSA	398MJ94SSA IV	Stock solution 100 IU/ml
Trypsin solution	In vitro	EN-005	0.05% enzyme solution without calcium and magnesium
75 cm <sup>2</sup> cell culture flask	Corning-Costar	430720	Canted neck flasks for trophozoite culture in TYI-S-33 medium

Transwell permeable supports	Corning-Costar	3470	0.4 µm polyester membrane, 6.5 mm insert in 24-well plate, growth area 0.3 cm <sup>2</sup>
24-well cell culture dish	Corning-Costar	3524	Clear polystyrene, treated for optimal cell attachment, sterilized by gamma radiation and certified non-pyrogenic
Complete Mini	Roche	11836 153 001	Protease inhibitor cocktail inhibits a broad spectrum of serine, cysteine and metallo-proteases. Final concentration 1 mM
Trans-epoxysuccinyl-L-leucylamido (4-guanidino) butane (E-64)	SIGMA-Aldrich	E3132	Cystein protease inhibitor, final concentration 40 µg/ml
paZO-1	Invitrogen	402200	IgG rabbit polyclonal antibody against a synthetic peptide in the middle region of the ZO-1 human protein
maEhCPADH112	Homemade antibody	Without/ Number	IgM mouse monoclonal antibody against 444-601 epitope located at C-terminal of EhCPADH112 <sup>21,27</sup>
FITC-goat anti-mouse IgM	Zymed	62-6811	Fluorescein isothiocyanate (FITC)-labelled goat anti-mouse secondary antibody
TRITC- goat anti-rabbit IgG (H+L)	Zymed	816114	Tetramethyl-rhodamine isothiocyanate (TRITC)-labelled goat anti-rabbit IgG secondary antibody.
STX2 Electrode	World Precision Instrument	102711	Consists of a fixed pair of double electrodes, 4 mm wide and 1 mm thick. Each stick of the electrode pair contains a silver/silver-chloride pellet for measuring voltage and a silver electrode for passing current. For use with EVOM
EVOM epithelial voltohmmeter	World Precision Instrument	12111	Use in resistance mode and maintain unplugged during TER measurements
Neubauer chamber	MEARIENFELD	610610	Hemocytometer
Leica TCS_SP5_MO	Leica	Without/number	Laser confocal microscopy with Leica microsystems CMS GmbH/ leica Las af Lite/BIN software
Vectashield	Vector Laboratories, Inc.	H-1000	Mounting medium for fluorescence
4',6-diamino-2-phenylindole (Dapi)	SIGMA	D-9542	0.05 mM final concentration
Bovine serum albumin (BSA)	US Biological	A-1310	0.5% final concentration for blocking solution